A GUIDE TO LABORATORY ACTIVITIES USING LIVE PLANARIA FOR SECONDARY SCHOOL ADVANCED BIOLOGY

A Field Report

Presented to

The Graduate Division

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Education

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by

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August 1965

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CHAPTER I

INTRODUCTION

An advanced biology course, offered as a twelfth-grade subject, has been added to the curriculum of the Marshalltown Community High School. The writer was given the responsibility for preparing and teaching the advanced biology course. It will be offered for the first time in the 1965-1966 school year.

Only those students that have had both general biology and chemistry are eligible to enroll in advanced biology. It was anticipated that the course should be challenging to the students, and worthy of the title Advanced Biology.

The divisions to be included in the advanced biology course are the following:

1. Adaptation for Living
2. Resources for Living
3. Maintaining the Organism
4. Maintaining the Species
5. Life on a Crowded Planet
6. A Closer Look at Species

The study of these divisions is prefaced by seven or eight days on the historical development of biology. This introduction is considered to be important to the student.

It also gives the teacher time to organize the laboratory and allows for the completion of any schedule changes by the students before the course proper is started.[1]

Regularly scheduled laboratory activities are a basic part of the course. This report presents detailed plans for a six-week laboratory unit using live planaria. The greatest difficulty in teaching a science course is to provide the students with laboratory experience that is challenging, provocative, and rewarding.

> Many students today find that the best instruction they receive in schools is found in the music, art, shop, and driver training classes. Why is this so? Primarily because in these classes they are permitted to act as musicians, artists, craftsmen, and drivers. A musician plays an instrument, an artist paints a picture, a craftsman builds a desk, and a driver drives a car. When it is time for their history, literature, or biology class, however, they are not generally permitted to behave as historians, critics, or biologists. In the biology class, for example, are they permitted to formulate their own experiments and discover something new to them? Generally not. Rather they are treated as if they were science historians. . . What we need to do for these students is to let them behave as biologists, and this can only be done through the use of the method of science.[2]

The investigator believed that the experiences offered to the students in the laboratory study of planaria make the

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[1]Phillip R. Fordyce, "The Advanced Biology Course," The American Biology Teacher, XXI (May, 1959), 169-170.

[2]Thomas G. Aylesworth, "Four Kinds of Thinking in the Biology Classroom," The American Biology Teacher, XXIV (December 1962), 598.

unit suitable for use in advanced biology.

I. THE PROBLEM

Planaria was selected as the organism to implement the course theme, "inquiry," because there is a known source of planaria in Marshalltown, and the laboratory study of planaria permits the student to do the things that biologists do. Planaria can be collected from their natural habitat and maintained in a small area with inexpensive care. Since the planaria offers unlimited experimental possibilities, a student working with such an organism would be challenged to accept the extended invitation to inquiry.

Statement of the problem. It was the purpose of this study to prepare a guide to laboratory activities using live planaria for secondary school advanced biology. The activities provided by the guide were designed for a six-week period.

Importance of the study. It is very important that the advanced biology course with its special function be carefully planned and that the laboratory activities be as productive as possible of desired learnings.

Bentley Glass believes there are two reasons for having students participate in laboratory activities. The

first reason is that it aids the student in gaining information and learning facts through the illustration of biological concepts by observing evidence from nature. The second reason for work in the laboratory is to impart to the students the nature of science, the spirit of scientific inquiry, and something of the methods of science--it is in the laboratory that the work of science is done.[1] Planaria can serve as a vehicle for accomplishing the primary functions of laboratory work.

Most high school and college laboratory workbooks use planaria, but for only one or two activities, their study being limited to the planaria as a typical flatworm. Therefore, a comprehensive laboratory guide for the study of planaria would be an aid to biologists if the above goals were to be achieved through the study of planaria. The guide to laboratory activities presented in this study utilizes the planaria as a laboratory animal which offers unlimited experimental possibilities to the high school student.

Limitations of the study. The study of planaria is a profitable and enlightening experience. However, the

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[1]Addison E. Lee, "The Block of Time Idea in Biology Laboratory Instruction," The American Biology Teacher, XXII (March, 1960), p. 135.

laboratory assignments need to be carefully planned to insure completion of the experiments in the six-week period allotted to this laboratory unit. The investigator has arbitrarily selected for use in the guide those activities pertaining to planaria which can be pursued advantageously in the specified time. The student may independently continue a line of investigation involving planaria following the conclusion of this unit.

II. PROCEDURE

Traditional high school sources were surveyed and found to be brief or lacking in their treatment of planaria. The American Institute of Biological Sciences, to improve biology education in the United States, organized the Biological Sciences Curriculum Study (BSCS), which has prepared textbooks, laboratory manuals, films, etc., for high school biology. Some of the new BSCS sources gave more attention to planaria, with one publication suggesting an interesting research problem concerning the amino acid content and regenerative properties of the planaria Dugesia dorotocephala.[1]

College texts, particularly zoology texts, were lib-

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[1]American Institute of Biological Sciences Biological Sciences Curriculum Study, Research Problems in Biology, Series Two (Garden City, New York: Doubleday and Co., Inc., 1963), pp. 117-123.

eral in their treatment of planaria; however, the college laboratory manuals were mostly minimal in their use of planaria.

Textbooks, reference books, scientific journals, and publications such as The American Biology Teacher, Science, American Institute of Biological Sciences, Turtox News, Chemistry, and The Welch General Science and Biology Digest, were the main sources of information used in preparing the guide.

General information, research information, and activities were adapted to fit the needs of the course and other activities were synthesized by the writer from the various sources of information. The material was then organized into a guide to laboratory activities using live planaria for secondary school advanced biology.

III. ORGANIZATION OF THE REPORT

The remainder of this field report is organized as follows:

Chapter II describes the advanced biology course in the Marshalltown Community High School and coordinates the laboratory guide, using planaria as the subject for a six-week experimentation unit.

Chapter III is a review of literature presented to establish a background for using planaria as a laboratory animal for secondary school advanced biology.

Chapter IV is a discussion of the work to be done in the laboratory and of the scientific method, followed by general laboratory instructions for the student.

Chapter V is, "A Guide to Laboratory Activities Using Live Planaria For Secondary School Advanced Biology" which is the topic of this field report.

The guide has the following exercises:

1. Field collecting
2. Care of planaria and observation
3. Ecology
4. The use of a key
5. Morphology
6. Regeneration
7. Respiration
8. Behavior of planaria--orientation

The activities are as unstructured as possible and are open-ended. Each activity is arranged with directions for the student, and pertinent information for the teacher is also given.

Chapter VI is a summary of the field report.

CHAPTER II

THE ADVANCED BIOLOGY COURSE AT MARSHALLTOWN COMMUNITY HIGH SCHOOL

Advanced biology is a second-year biology course often offered for the student interested in biology or applied biology as a future profession. However, if future use as a profession is the primary goal, the course is apt to end in failure because the subject matter emphasis may be too great. The goals should be to develop independence of thought, to develop the ability to ask questions, and to stimulate the development of methods in designing experiments or observing to obtain evidence which will aid in answering the questions posed. Above all, the student must be encouraged to use his ingenuity. Prerequisites for taking advanced biology are systematic biology and chemistry. It is recommended that students have had mathematics through advanced algebra. The course is two semesters in length and meets a minimum of seven fifty-five minute periods a week. Three periods in the classroom and two double laboratory periods are planned, but this can be modified if necessary.

The text selected was Design For Life[1] as it is

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[1]Richard F. Trump and David L. Fagle, Design For Life (New York: Holt, Rinehart and Winston, Inc., 1963).

functional in approach and flexible in arrangement so that altering the sequence of presentation can be accomplished without sacrificing time or scholastic quality. In the preface of their text, Trump and Fagle wrote:

> When Louis Agassiz first met one of his natural history classes in 1873, he remarked, "I shall never make you repeat what you have been told, but constantly ask what you have seen for yourselves."
>
> This was a courageous statement, and perhaps not completely practical. If tempered with wisdom, however, this philosophy can be a wholesome challenge to both the teacher and student.
>
> In this book we attempt to think through with the student the meaning of biology. In a limited way, then, the student has an opportunity to "see" for himself. If he participates in the thinking-through process, he need not merely repeat what he has been told. He can create the meaning of biology for himself. We say this with a good deal of humility because we realize that the teacher, not the book, is the most important influence in directing a student's thinking.[1]

The division and chapter titles shown in the course outline are taken from the textbook with some rearrangement in sequence. The time schedule shown in the course outline totals twenty-eight weeks, which permits extending the time allowed for a division, especially laboratory time, and the possible introduction of a student-instigated activity not in the course outline. But, it is the investigator's plan that the greater portion of time not allocated be utilized for pursuit of the students' investigation or research that

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[1] Ibid., p. vii.

they have selected and planned.

I. PURPOSES OF THE COURSE

Purposes of the advanced biology course were:

1. To further stimulate and encourage creative thinking and doing--to foster in the student a "sense of inquiry".
2. To provide opportunities for the capable students to extend into areas beyond the suggested limits.
3. To increase the depth of concepts gained in general biology by investigating more complex problems.
4. To maintain interest created in biology by minimizing the time lapse between 9th or 10th grade and college enrollment.

In answer to a growing demand, the BSCS in 1962 began preparing an advanced biology course for experimental use during the school year 1962-1963. The committee preparing the course agreed that:

> the primary goals of an advanced biology course should be to further the students' understanding of scientific inquiry including: the statistical analysis of data, the reading of scientific literature, and the exploration of conditions which influence the progress

of scientific investigation.[1]

II. COURSE OUTLINE

The following is an outline of the course:

I. Introduction — 3 weeks

- A. Historical development of Biology
- B. Adaptations For Living
  - 1. Biological Design
  - 2. The Design at Work
  - 3. Understanding the Design

II. Resources for Living — 7 weeks

- A. The Chemistry of Life
- B. Organizing Chemical Activity: The Cell
- C. The Self-Feeders
- D. The Dependent Feeders
- E. The Digestive Process
- F. Order in the World of Life: Classification
- G. Life and Its Environment

III. Maintaining the Organism — 7 weeks

- A. The Transport Problem
- B. Closed Circulation: Indirect Delivery
- C. Energy Transfer

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[1] American Association for the Advancement of Science, The New School Science, A Report to School Administrators on Regional Orientation Conferences in Science (Washington, D.C.: American Association for the Advancement of Science, n.d.), p. 33.

D. Construction and Repair

E. Coordination: Chemical Control

F. Coordination: Neural Control

G. Effective Behavior

IV. Maintaining the Species 6 weeks

A. Understanding Reproduction

B. A Review of Heredity

C. Heredity: Variable Patterns

D. Development

E. Unity of Life: Evolution

V. Life on a Crowded Planet 2 weeks

A. The Nature of Interdependence

B. Man and Interdependence

C. Putting Ecology to Work

VI. Closer Look at Species 3 weeks

A. E.coli, Bacterium

B. Zea mays, a Plant

C. The Honeybee

D. Man and the Future 28 weeks

III. LABORATORY EXPERIENCES

Advanced Biology in this course has a functional approach. In modern biology the molecular, biochemical, and physiological aspects of life are favored over anatomical

detail; however, biology is the study of life and living things. The complete organism and its behavior are also considered in some detail. The interrelationship of the sciences is of great importance and is apparent particularly in the laboratory exercises. Wide reading is essential and each student is required to prepare four journal article summaries and one reference text summary each semester.[1]

There is great emphasis on laboratory and laboratory techniques for meeting a problem--not for technique's sake, but for the student appreciation of the way in which a scientist works and for the stimulation of overcoming problems of technique. The primary objective of the field report is to prepare a laboratory unit using one organism to meet the objectives above. The students will prepare their own media, stains, and reagents. Sterilizers, incubators, a bacteria colony counter, an analytical balance, chromotography supplies, and a microtome are part of the laboratory equipment, and the students are expected to gain some facility in their use.

The laboratory exercises are formal in part, but whenever possible are unstructured, and the student is responsible for collecting and improvising equipment to successfully

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[1]Phillip R. Fordyce, "The Advanced Biology Course," The American Biology Teacher, XXI (May, 1959), 169-170.

conduct the experiment.

The areas selected for study in "A Guide To Laboratory Activities Using Live Planaria For Secondary School Advanced Biology" are the following: field collecting, care of planaria and observation, ecology, use of a key, morphology, regeneration, respiration, and taxic responses to stimuli in the planaria's environment.

The laboratory unit using live planaria will be introduced near the end of the second course division "Resources for Living." It may be advantageous to collect the planaria sooner to insure an adequate supply because the unit will not be begun until mid or late November.

CHAPTER III

THE USE OF PLANARIA AS A LABORATORY ANIMAL

The purpose of the review of literature presented here is to establish a background for using planaria as a laboratory animal for secondary school advanced biology.

The flatworms are the simplest known animals to exhibit many of the characteristics found in the higher animals, including man.

> The flatworms, as illustrated by the planarias, are advanced over two-layered animals in a number of important characters which are possessed by all higher animals. The flatworms are the first animals to have specialized anterior and posterior ends and dorsal and ventral surfaces. They are the first to have a definite head with a concentration of sense organs and the development of a central nervous system. And they are the first to make extensive use of a third layer of cells, the mesoderm, which, either by itself or in combination with ectoderm or endoderm, gives rise to organs and organ systems.[1]

Therefore, the flatworms are studied to some extent in nearly all biology courses because of their unique place in the natural relationship of animals.

Some free-living flatworms, class Turbellaria, have remarkable powers of regeneration. Regeneration is most common in the order Tricladia which includes planaria. Saunders in 1931 wrote, "Lost parts are easily regenerated

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[1]Ralph Buchsbaum, Animals Without Backbones, Second Edition (Chicago: The University of Chicago Press, 1948), p. 120.

in the Tricladia and the group is a favorite one for experimental work on regeneration."[1] According to Buchsbaum, some planaria can regenerate complete worms from very small pieces whereas the phenomenon is not observed in the parasitic flatworms.[2]

The Twentieth Symposium of the Society for the Study of Development and Growth met in Williamstown, Massachusetts, June 12-14, 1961, and was devoted to surveying progress in regeneration. Eminent scientists made reports of their scientific research on regeneration both completed and currently in progress. The first three chapters of the book containing the published reports of the symposium pertain to the newer and special aspects of regeneration in invertebrate animals. The third chapter by Etienne Wolff of Paris, France, is a summary of the studies reported by investigators concerned with neoblastic regulation and tissue interaction in planaria.[3]

The current literature shows that planaria is the

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[1]L. A. Borradaile, F. A. Potts, L. E. S. Eastham and J. T. Saunders, The Invertebrata, Second Edition (Cambridge: The Syndics of the Cambridge Univ. Press, 1935, 5th Printing 1951), p. 213.

[2]Buchsbaum, op. cit., p. 125.

[3]Dorothea Rudnick, Editor, Regeneration, (New York: The Ronald Press Company, 1962), pp. 53-81.

subject of an increasing amount of research. Zoologists have been joined by psychologists and biochemists in much of the recent and current research on planaria.

It has been established that planaria can be trained or conditioned; also, that when cut in half and each portion allowed to regenerate into a complete worm, both worms were still trained. Ernhart had trained planaria to solve a maze to reach a dark sheltered area. The trained worms were cut in half and allowed to regenerate forming two new worms. When tested, the group of worms that had grown new heads remembered as well as the group that had grown new tails. He concluded that in planaria learning retention, of motor skills at least, is on the cell level rather than centralized in the brain.[1]

McConnell and group trained the flatworms to respond to a flash of light by administering an electric shock in conjunction with, but an instant after, the light flash. The conditioned worms were cut in half and allowed to regenerate into complete worms. Both new worms when tested were found to be trained. Conditioned planaria were then cut into three or more pieces and allowed to regenerate into complete worms. When tested all the new, regenerated worms exhibited

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[1] American Biology Teacher, "Memory", XXIII, March 1961, 136.

about the same degree of memory. It was concluded that conditioning caused a chemical change, perhaps in the brain, and this change affected all parts of the body.[1]

Reeva Jacobson, working in McConnell's laboratory, cut planaria in half and conditioned the heads before any regeneration could take place.[2] She then allowed the conditioned heads to grow new tails which, when growth was complete, were severed and allowed to regenerate new heads, resulting in completely reformed organisms. The total regenerates, as they were called, were tested for retention of the original conditioning. The total regenerates did not display the degree of retention that the original animals had shown; however, the amount of retention was of such significance that to the researchers their hypothesis, i.e., that conditioning caused a chemical change, was validated.

Roy John and William Corning at the University of Rochester were following the same line of research, and they, too, concluded that a chemical change in the cells was responsible for memory retention.[3] They further formed the

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[1]Thomas Aylesworth, "Scientists and Flatworms," Current Science, XLIX (January 27-31, 1964), 129-131.

[2]James V. McConnell, "Memory Transfer Through Cannibalism in Planarians," Journal of Neuropsychiatry, III Supplemental (August, 1962), s44-s45.

[3]Ibid., 465-468.

hypothesis that ribonucleic acid (RNA) might be the chemical suggested by the experiments. Planaria were then conditioned, halved, and allowed to regenerate in a weak solution of ribonuclease, an enzyme that breaks down RNA. After regeneration to completed worms, the head sections remembered but the tail sections did not. It seemed, then, that an inadequate supply of RNA inhibited memory retention, but why the heads remembered in spite of the low RNA level was still not known.

An article in Chemistry, reporting on the work of McConnell and group, state that "once this concept that memory retention can be the result of a chemical process was established, a whole new field of experimentation was opened."[1] The article continues with an account of other research--some just concluded, some currently in process, and some contemplated--all designed to investigate the chemical and neural aspects of the memory system of planaria.

The study of planaria is not limited to areas connected to regeneration and memory. Jennings writes of interesting research concerning the behavior of planaria under various ecological and natural conditions and

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[1]"Chemical Aspects of Memory," Chemistry, XXXVII (July, 1964), 23-24.

situations.[1] One condition that Jennings reports is the drying up of the planaria's habitat. A worm was isolated in a drop of water and observed continually as the water evaporated, until death occurred. This experiment was repeated a number of times, and Jennings discusses the unique behavior exhibited by the worms while attempting to cope with the condition, climaxed in each case by turning the head under the body and dying in this position. A situation discussed by Jennings is the inverted or upside down position. All planaria, even pieces, go through the same righting reaction in this situation which is to twist the body until some part of the anterior ventral surface comes in contact with the bottom of the container and the remainder of the worm is righted by adhering gradually front to back.

Stringer wrote:

> The Turbellaria or free-living flatworms are among the most interesting of the simply organized animals because of the remarkable variety shown in their reactions and behavior. . . .[2]

and a few pages later wrote

> . . . the larger planarians are especially valuable for study in laboratories where attention is given to animal behavior. Certain forms afford excellent

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[1]H. S. Jennings, Behavior of the Lower Organisms, 4th printing, (New York, New York: Columbia University Press, 1931), pp. 242-247.

[2]Henry Baldwin Ward and George Chandler Whipple, Fresh Water Biology, (New York: John Wiley and Sons, Inc., 1918), p. 323.

training in exactness of observation.[1]

Fraenkel and Gunn in discussing klino-kinesis cite for illustration Ullyott's (1936) work with the planarian Dendrocoelum lacteum.[2] The klino-kinetic response is the increase or decrease in the r.c.d. (rate of change of direction) as the intensity of the stimulus is increased or decreased. The change of direction is random turning, and when the rate changes, the linear velocity remains constant. The basal r.c.d. for the planaria studied was 103 degrees each minute, but when stimulated by a strong light, the planaria's r.c.d was 160 degrees for each 30 seconds.

Fraenkel and Gunn cited experiments using arthropods for illustrating tropo-taxis because the eyes of arthropods are the most suitable for tropo-tactic behavior and are also suited for experimental elimination--painting over one or both eyes.[3] However, it was stated that the planaria's eyes are only slightly less suitable in both ways mentioned, and that the modern views on tropo-taxis are due in large measure to Taliferro's (1920) work using

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[1]Ibid., p. 331.

[2]Gottfried S. Fraenkel and Donald L. Gunn, The Orientation of Animals, (New York: Dover Publications, Inc., 1961), pp. 43-57.

[3]Ibid., p. 89.

Planaria maculata. The tropo-tactic response requires that two or more receptors are involved and that the animal maneuvers in such manner that the receptors are stimulated equally as the animal then moves either directly toward or away from the stimulus.

Planaria have been used for investigating tactic responses to other stimuli such as gravity, chemicals, and currents in water, but these will not be discussed in this review.

Brenneman indicates that the class name Turbellaria was applied to the free-living flatworms by Ehrenberg in 1831, because when he, Ehrenberg, ovserved them, they created a turbulence in the water around them.[1] The agitation was caused by the cilia on the body of the flatworm.

Concerning reproduction, morphology, and taxonomy, Hyman is one of the foremost experts on planaria. Authors that write about the flatworms cite Hyman extensively. This review makes no attempt to discuss Hyman's work; however, the identification key presented as a part of this field report has been adapted largely from Hyman.[2] Information on collecting and maintaining planaria from

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[1]W. R. Brenneman, Animal Form and Function, (Chicago: Ginn and Company, 1959), p. 146.

[2]Henry Baldwin Ward and George Chandler Whipple, Fresh Water Biology, (New York: John Wiley and Sons, Inc., 1959), pp. 326-334.

Hyman is also used in this report.

Planaria can be collected from their natural habitat and are easily maintained in the laboratory. Also, most authors, when writing of field collecting, mention the different phases of environmental studies that may be pursued.

CHAPTER IV

THE LABORATORY

The material presented in this chapter is for students and applies to all laboratory activities in the Advanced Biology Course. It is the student's "invitation to inquiry," followed by general laboratory instructions.

This section will be discussed with the students during the first laboratory session of the year, coordinated with an unstructured laboratory activity, a "black-box experiment." In this experiment the students find a box at their station and are asked to identify the object in the box. Paper is provided for recording data collected from their observations in attempting to identify the object. They can determine the state of the object, the approximate shape, the approximate size and hardness or softness. In an exercise of this type, the student's mental powers and imaginations are taxed with a minimum of direction.[1]

I. INTRODUCTION

A laboratory is the place in which the work of science is done. Success depends in a large measure upon the student's attitudes toward his work and upon his ability

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[1]Alfred Novak, "Scientific Inquiry in the Laboratory," The American Biology Teacher, XXV (May, 1963), p. 344.

to work independently. Because the student does not have the background of reading and experience in scientific research, some activities will be of a formal nature, a completely structured activity in which the problem, procedure, equipment list, directions, and even results may be given.[1] When possible, however, unstructured laboratory activities will be pursued, and the student will be extended an invitation to inquiry.

In most unstructured activities, after the area for study has been designated and introduced by the instructor, the student must function as a scientific investigator. He is then responsible for defining the problem or problems, formulating a tentative solution or hypothesis, devising methods or procedures and assembling equipment, collecting and evaluating data. Some activities may be in areas not discussed in the text and will necessitate a search of supplemental literature. The student must be alert and learn to observe details as well as general relationships.

The Scientific Method

There are many so-called scientific methods, all having the common goal of finding a solution to a defined or definable problem. The different methods are usually

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[1] Ibid., p. 343.

related to the type of investigation. If the investigation involves testing such as for blood type, fat content, or the strength of drugs, the technical method is used. A scientist using the technical method is not concerned with making new discoveries or trying new procedures, but (1) follows an outlined procedure, (2) makes accurate observations and (3) records and reports all findings.

The research method is used to prove or disprove theories. The research scientist is free to try new and unique procedures in man's endless quest for knowledge of the world in which he lives. But, while the technical method is the more widely used, the research method is usually implied when referring to the scientific method.

Scientific methods range from doing one's best with the facilities and equipment available, to complicated outlines with as many as ten steps to be followed closely.

The major steps of the scientific method are as follows:

1. Recognizing and defining the problem
2. Forming an hypothesis
3. Collecting information, testing, and organizing data
4. Drawing conclusions

Successful completion of an experiment forces use of the scientific method.

Use of the Scientific Method.

The scientific method adapts itself easily to the solution of many problems in daily living as well as those encountered in the laboratory and field. The student should approach each problem, fact, laboratory or field observation with a curious but open mind so that he may collect information and data accurately, from which to formulate logical conclusions. The activities of part one are designed for the student to: (1) practice the scientific method by following the steps and procedures outlined previously, (2) become familiar with the facilities, equipment, and techniques of the biology laboratory and (3) learn something of the history of biology and acquire knowledge of biological principles and concepts that are essential for the serious study of biology.

The scientific method, as mentioned above, is not foolproof. Experiments which end in failure are encountered by students and scientist, alike. But many times the scientist, and perhaps the student, learns as much or more from things which do not work as from those that do.

Morris Goran, writing in Chemistry, quoted Kepler:

> how many detours I had to make, along how many walls I had to grope in the darkness of my ignorance until I found the door which lets in the light of truth.[1]

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[1]Morris Goran, "Scientists Also Make Mistakes," Chemistry, XXXIIV (April, 1964), 28.

Goran also wrote of mistakes, some because of negative beliefs; for instance,

> Lord Rutherford said that man would never tap the energy in the atomic nucleus; the first atomic bomb exploded a few years after his death. Muller, a leading physiologist of the nineteenth century, asserted that the speed of the nerve impulse would never be determined; six years after this was said, Hermann Helmholtz measured the speed of nerve impulse in a segment of frog nerve only a few inches long. . .[1]

The Aims of Science

H. Bentley Glass, BSCS Chairman, stated in The New School Science:

> There are two major aims in studying any natural science. One aim, the lesser in importance, is to become acquainted with the significant scientific facts upon which rest the major concepts and theories of science. These are the ideas that have so profoundly altered our views of man's place in nature and have so tremendously enlarged human powers over the forces and resources of nature. In biology, this objective also includes a firsthand acquaintance with living organisms and the outstanding features of their lives.
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> The other aim is indispensable to young scientists and non-scientists alike--to everyone who hopes to participate intelligently in the life of a scientific age which so constantly demands difficult decisions and real wisdom. This second objective is to know what science really is--to recognize its spirit and to appreciate its methods. Science is not magic, and a scientific civilization surely will not endure if most people of intelligence regard science as a sort of magic. It is a way--or a composite of many ways--of finding out reliable, confirmed knowledge about all natural phenomena. It is compounded of the observations of the human senses

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[1]Ibid., p. 29.

and the inferences and deductions that can be derived from such experiences.[1]

II. GENERAL INSTRUCTIONS FOR THE STUDENT

Materials and Equipment

The student must supply himself with the following materials and equipment: laboratory manual, biology laboratory notebook, drawing pencils, towel or soft cloth, six-inch ruler, razor blades, and manilla folders.

All other laboratory equipment will be supplied by the school and made available to the student as needed. Specimens will be supplied by the school or collected by the students while on field trips.

Each student will be assigned a permanent work area with storage space which has the following basic equipment: dissection materials, slides, cover glasses, lens paper, bunsen burner, test tubes, beakers, bottles, inoculating needles, slide forceps, test tube brushes, rubber tubing, and stirring rods. Other equipment is available, and the student is encouraged to work independently whenever the laboratory is open and the instructor is present.

At the end of the laboratory period the student will

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[1]American Association for the Advancement of Science, The New School Science, A Report to School Administrators on Regional Orientation Conferences in Science (Washington, D.C.: American Association for the Advancement of Science, n.d.), p. 26.

return equipment, materials, and specimens to their proper places. The table and the floor area around the table must be cleaned. Students will hand in accounts of their work as required by the instructor, usually at the conclusion of each investigation. Accounts will include a description of experiment, problems, procedure, and conclusion.

Drawings

Accurate drawings are important in science. They are a stumulus to critical observation, and drawings enable others to understand or duplicate the work being discussed. The drawing is made while observing the specimen or apparatus and not from memory.

A sharp pencil (3H or 4H hardness) should be used for all labeling and drawing. The magnification of the drawing must be noted as 100x if magnified 100 times or $\frac{1}{4}$x if drawn one-fourth actual size. The student should strive for neatness and accuracy; draw what is seen, not what others see or imagine because a drawing is a means to an end--not an end in itself. Some drawings will be required. The student should make additional drawings if they in any way add clarity or demand that the observer be more critical in his observation.

Answering Questions

When a question occurs to the student, he should (1) attempt to answer it from his fund of knowledge by careful thought and further observation, (2) attempt to answer his question by reading literature related to the subject, or (3) ask the instructor for assistance. The student should follow this order, and the instructor should be consulted only after other resources are exhausted. The instructor may give an answer to the question or direct the student to an activity which will enable the student to find the answer.

CHAPTER V

A GUIDE TO LABORATORY ACTIVITIES USING LIVE PLANARIA FOR SECONDARY SCHOOL ADVANCED BIOLOGY

Planaria are aquatic free-living flatworms in the phylum Platyhelminthes which includes tapeworms and liver flukes. These flatworms, planaria, can usually be found in ponds and streams and collected from vegetation, the undersides of rocks and submerged logs. After collection, planaria can be easily maintained in earthenware, glass, or enameled pans.

Planaria are widely used laboratory animals which are studied to some extent in most secondary school and college biology classes because of their place in the natural relationship of animals since they possess mesoderm and cephalization early in the phylogenetic series. Research zoologists for a century or more have used planaria as the subjects of intensive study in the areas of growth, development, and regeneration. Since approximately 1950, zoologists have been joined by psychologists and biochemists in the investigation of the chemical and neural aspects of the memory system of planaria.

Six weeks will be devoted to the study of planaria which will necessitate periods of daily laboratory work.

The unit will consist of certain structured and unstructured activities or experiments designated by the instructor. The student is encouraged to devise other experiments which may be pursued independently.

The exercises are presented in the following order:

1. Field collecting
2. Care and observation
3. Ecology
4. Use of a key
5. Morphology
6. Regeneration
7. Respiration
8. Behavior of planaria--orientation

EXERCISE I. FIELD COLLECTING

Directions for the Student

Activity 1. Plan a field trip for collecting planaria from their natural habitat and collect specimens for observation and use in future investigations. It may be necessary to make more than one collecting trip to obtain a sufficient number of planaria.

Return to the laboratory any specimens collected and place in an appropriate container. Mark on the container place and date of collection and record other useful

information.

Hand in a report of the field trip. Include in the report a list of equipment taken, indicating what equipment was used and what might have been needed; where the planaria were found (near the surface, on vegetation, or on rocks); and kind of bait used, if any.

Information for the Teacher

Most references when discussing planaria indicate that planaria are easily collected from ponds and streams by baiting with raw liver. However, it has been this investigator's experience that it is difficult to locate planaria in some streams and ponds and that some planaria are not attracted to beef or liver. He has collected planaria from the underside of rocks that were lying between pieces of bait that did not attract the planaria.

The cemetery pond in the Marshalltown area is a good source of planaria. Planaria will be first sought from a source other than the cemetery pond, but if the search is unsuccessful a trip will be planned to this known source of planaria. The planaria in the cemetery pond are Dugesia tigrina and are best collected from the underside of rocks.

David Fagle, a co-worker in Marshalltown, has collected a larger species of planaria from Rock Creek Lake

near Newton. Mr. Fagle has also collected planaria from the lake in Union Grove State Park near Gladbrook.

Students should be cautioned to replace rocks and, in general, maintain the habitat.

A reference for methods of collecting and maintaining different species of planaria and other invertebrates is Culture Methods For Invertebrate Animals.[1]

EXERCISE II. CARE OF PLANARIA AND OBSERVATION

Directions for the Student

Activity 1. During the time planaria are being investigated, each student is responsible for the care and maintenance of his supply of planaria.

Students will keep a log of the number of planaria collected, kind of culture container, food, and when fed; mortality rate and possible cause; source of water in container and time and date changed; and other information which is deemed important.

Activity 2. In future activities the student must have dexterity in the handling and manipulation of planaria. He must devise means for observing and displaying planaria

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[1]James G. Needham et al (ed.), Culture Methods For Invertebrate Animals, (New York: Dover Publications, Inc., 1937).

from all sides. Actual photographs of planaria might be helpful.

For practice in manipulation and observation, the following exercise, modified from a first year biology laboratory manual, is presented:

> Place a single planarian in a watch glass and cover it with a drop of aquarium water. Observe its movements carefully, using a hand lens, or a dissecting microscope.
>
> Describe the way the animal moves. _____
> _____
> _____
>
> How does its movement differentiate the <u>anterior</u> from the <u>posterior</u> end of the body? _____
> _____
>
> Touch the planarian with the end of a dissecting needle. Is there any response? _____
>
> What sense organs are seen near the anterior end? _____
> _____
>
> The projecting lobes on each side of the head region are called <u>auricles</u> and are believed to be sensitive to chemicals dissolved in water. Pour a few milliliters of dilute acetic acid into a small jar. Dip the end of a dissecting needle into the acid. When the planaria is close to the edge of the drop of water in the watch glass, dip the acid-tipped needle into the edge of the water.
>
> Does the worm respond positively or negatively to this stimulus? _____
>
> In a similar way, test its response to a tiny amount of ethyl alcohol. Result? _____
> _____
>
> With a small pipette, remove most of the water about the flatworm. Add ten drops of 2 per cent Magnesium sulfate solution.
>
> What is the result? _____
> _____

Make a sketch of the planaria. Label the head, anterior and posterior ends, eyespots, and auricles.[1]

When the planaria is touched with the end of a dissecting needle, does it make any difference where the planaria is stimulated? _____

Activity 3. Observing planaria which have been starved several days feeding on a colored food is an aid in identifying living specimens. Liver or raw beef or blood clots which have been puddled in a paste of carmine or carbon in water will suffice. Specimens fed in this manner may be used for preparing whole mounts. However, the main value of this activity is in the observation of the manner in which planaria capture and ingest live food.

The following quotation describes such experimentation:

> Planarians may be observed eating live food if they are offered freshly hatched brine shrimp Artemia salina. Observation of feeding is facilitated if the planarians are kept in a dish with a thin, flat bottom, such as a Petri dish, and the dish is laid on a mirror. Brine shrimp provide a satisfactory food for maintaining cultures of planarians for long periods.....
>
> Dried brine shrimp eggs may be kept on the shelf and caused to hatch as they are needed.....
>
> The brine shrimp eggs are caused to hatch by sprinkling a quarter teaspoonful of eggs on the surface of a solution of one tablespoonful of sodium chloride in a quart of water, in a broad, shallow dish. In

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[1]William H. Gregory, Laboratory Manual for Biological Science for High School, (Chicago: Ginn and Company, 1965), p. 119.

> about twenty four to forty eight hours, depending on the temperature, the freshly hatched brine shrimp may be caught in a very fine net and washed free of salt, or pipetted into a large volume of fresh water and re-concentrated by making use of their tendency to gather in the most strongly lighted portion of a container. . . .
>
> For best results in demonstrating feeding, it is recommended that the animals not be transferred to different containers of culture water just before food is offered. These animals are very sensitive to slight chemical changes in their environment, and respond to food more readily if they have been allowed to become adjusted to their surroundings for a couple of hours. Worms freshly transferred are usually hyperactive, often passing over food and ignoring it, even when the glassware seems really clean and the water quite safe for them. Excessive handling may also affect the worms' responsiveness. Worms accustomed to their containers usually remain quietly in one place for long periods until stimulated by touch, light, or the presence of food.[1]

Add a few brine shrimp nauplii to the dish containing the planaria and observe closely. Record observations.

Make a separate report on brine shrimp, including calculations showing the salt concentration of hatching water.

Activity 4. Righting reaction. In a small amount of water, place a planaria ventral side up and observe closely. Repeat, with a different planaria each time, five or more times. Make sketches of the responses of the animals to the situation and include with your report.

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[1]Helen Forrest, "Observing Planarians Feeding on Brine Shrimp, Turtox News, XLI (January, 1963), 34-35.

Activity 5. Determine the size--length, width, and thickness--and weight of a planaria. Use as many planaria as you think necessary to validate the data.

Activity 6. Do planaria swim? Observe the planaria's ventral surface under the microscope. How can you do this? Sketch an optical cross-section, and a whole ventral surface of the planaria showing areas which may aid in locomotion.

Information for the Teacher

Activity 1. Planarians of practically any species may be kept successfully in the laboratory in glass or crockery containers or enameled pans. These should be darkened by means of suitable covers. Treated city waters are not very suitable, but most species will live in such water for a considerable time. Spring or well water is desirable.

> . . . They are fed two to three times a week on beef liver (pig liver is not suitable). Before feeding, the water in the pan should be lowered to a depth of several inches. The pan is then covered and left undisturbed for 2 or 3 hours, after which the liver is removed, the pans thoroughly rinsed, and filled with fresh water. Even if the animals are not fed, the water should be changed two or three times weekly as planarians are very susceptible to fouling of the water. All food fragments should be carefully removed. . . .[1]

Planaria collected from springs or running water require more care than those collected from ponds. The investigator kept approximately thirty planaria, Dugesia tigrina, collected

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[1]James G. Needham, op. cit., p. 155.

from a pond, for six weeks in a pint jar and the water was never changed. The jar was filled with pond water when the planaria were collected, and then it was left open, near a north exposure to light. Tap water was added to maintain a constant water level.

Activity 2. The planaria moves in a gliding fashion, with the anterior end forward, due to activity of cilia on the planaria's ventral surface.

Does the planaria respond when touched with the dissecting needle? Yes.

Sense organs near the anterior end are eyespots and auricles.

The worm responds negatively to acetic acid and ethyl alcohol and moves or glides away.

When magnesium sulfate solution is added the planaria acts as though anesthetized, movement ceases. magnesium sulfate, epsom salt, is an anesthetic which is used to relax planaria in investigations where immobility is an asset.

Activity 3. Planaria are in the class Turbellaria, subclass Coelata, which is divided into orders according to the branching of the intestine or gastrovascular cavity. Planaria are small so that colored food, if ingested, will exhibit the shape or branching of the intestines. The

brine shrimp nauplii are orange, and the branched intestine may be observed.

The brine shrimp are swimming forms that could easily escape planaria. But Forrest indicates that the shrimp soon settle to the bottom becoming entangled in the trails of mucous left by the worms.[1] They are then easy prey for the planaria which probably sense their presence by chemoreceptors.

> In using a mirror to observe feeding, a hand lens may be employed to provide the necessary magnification, or Petri dish and mirror may be placed, slightly tilted, on the stage of a dissecting microscope. All details of the feeding process will be clearly visible in an unobstructed reflection of the undersides of the animals. Planarians react negatively to bright light, but most worms kept without food for a couple of days will feed normally if observed by ordinary room illumination. Under these circumstances, a feeding planarian will be seen to extend its pharynx, bend it around sinuously like an elephant's trunk, expand the end like a tiny funnel, and briskly pull in the Artemia as if sucking them through a straw.[2]

Activity 4. The righting reaction of planaria is usually the same in all cases. The body is twisted until the anterior ventral surface is brought into contact with and adheres to the substrate. Then the rest of the animal is pulled to its normal position.

Activity 5. To determine size, place the planaria on

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[1]Helen Forrest, op. cit., p. 35.

[2]Ibid.

a transparent plastic metric rule and observe length and width, thickness can be estimated. For determining weight, measure or weigh the amount of water in a beaker or other container. To the water add a number of planaria which have been placed on a paper towel, or filter paper, for a few seconds. Weigh again. The additional weight divided by the number of planaria will give the average weight of one animal. The added volume divided by the number of planaria will give the average volume.

Activity 6. Planaria do not swim. They move by action of cilia on their ventral surface. They can "walk" on the surface film of water, but if they lose contact with the surface, will fall to the bottom.

To observe ventral surface, place the animal on a cover slip, then invert and place cover slip on a slide, and the planaria will be ventral side up. Use magnification necessary to see cilia.

EXERCISE III. ECOLOGY

Directions for the Student

Activity 1. Describe the field environment of planaria which can be determined without using apparatus.

Activity 2. In this activity you will study some of the physical factors of the planaria's environment or habitat. Check the planaria's natural habitat for pH, oxygen content, and carbon dioxide content. These factors should be checked on both a sunny day and a cloudy day. If possible, check morning, noon, and evening. If it is inconvenient to return to the natural habitat, simulate the natural conditions of the habitat in the laboratory and check the required factors.

An acid solution is one containing an excess of hydrogen (H^+) ions. A basic or alkaline solution is one containing an excess of hydroxyl (OH^-) ions.

The pH scale is a system devised by chemists for indicating the degree of acidity or alkalinity of a solution. A pH of 7 is neutral. Numbers 0 to below 7 indicate acids and numbers above 7 to 14 indicate bases. The smaller the number, the stronger the acid; the larger the number, the stronger the base.

Your background in chemistry will help you determine ways for identifying pH.

A method for determining oxygen content of water, and a method for determining carbon dioxide content of water is given, as follows:

THE WINKLER METHOD FOR MEASURING OXYGEN IN PARTS PER MILLION DISSOLVED IN THE WATER[1]

1. Fill your oxygen sample bottle (100 to 150 cc. capacity) with water to be studied. Do it without bubbling. Siphon with a tube from a bucket dipped into the pond.
2. Add 10 drops (about 0.5 cc.) manganous sulfate solution. To make solution put 480 grams of manganous sulfate ($MnSO_4:4H_2O$) in 1 liter of water. This keeps indefinitely.
3. Add 10 drops (about 0.5 cc.) potassium hydroxide-potassium iodide solution. To make up solution add 500 grams sodium hydroxide or 700 grams potassium hydroxide to a liter of water. It gets hot. Then add 135 grams sodium iodide or 150 grams potassium iodide. Keep in rubber-stoppered bottle.
4. Put top on bottle and mix with three wrist motions. Keep bubbles out if possible.
5. Wait one minute in fresh water. (15 minutes in salt water).
6. Take out top and add 15 drops sulfuric acid (about 0.5 cc.). Put top in and mix with wrist motion. This is concentrated sulfuric acid obtainable from any service station where it is used for batteries. This burns holes in clothes, metal, skin if not washed off.
7. The sample bottle should be yellow due to the formation of iodine. There is an equivalent amount of yellow iodine present for dissolved oxygen originally present. If there is no oxygen, the bottle will be clear; if there is much oxygen, the bottle will be bright orange. Thus you can tell something roughly without the next step. At this point the sample or samples may be kept for a day or two. One can do this part outdoors and bring back to school for the rest.

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[1]Howard T. Odum, "Ten Classroom Sessions in Ecology," The American Biology Teacher, XXII (February, 1960), 71.

8. With a measuring device of some sort (graduate cylinder, pipette, glass with a mark on it) measure out 100 cc. of the yellow solution.
9. Add several drops of fresh starch solution, enough to give a black or blue-black color. Starch solution can be made by boiling a tablespoon of mashed fresh potatoes in a cup of water for 5 minutes and filtering through a fine cloth. It filters slowly, but one only needs a few drops. One can also use corn starch, crackers, or scraps of notebook paper in a pinch.
10. Then titrate with hypo solution. One does this by adding drop by drop hypo solution from a burette or graduated pipette until the solution changes from black through blue until clear. The number of cc. of hypo solution added is the number of parts per million of oxygen dissolved in the original solution. The hypo solution is made by adding 3.102 grams sodium thiosulfate (hypo from photo shop) to a liter of water. In refrigerator this keeps for several weeks. The hypo and the starch have to be made up fresh when one starts work after a period of time.

Abbreviated Table of Saturation Values for Oxygen in Parts Per Million (ppm)

	Temperature, Degrees Fahrenheit			
	32	50	75	90
Oxygen in fresh water	14.2	10.8	8.2	7.2
Oxygen in salt water, 3.5%	11.3	8.7	6.7	4.1

A METHOD OF MEASURING CARBON DIOXIDE[1]

Carbon dioxide (CO_2) is a compound of great importance to life. Living organisms constantly produce it during respiration, and all green plants absorb large quantities of it when exposed to light. Thus, a method for measuring carbon dioxide quantities is a useful tool in biology. This exercise provides a method that requires only simple equipment.

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[1]Chester A. Lawson, Laboratory and Field Studies in Biology (Teacher Edition), A Sourcebook For Secondary Schools, (New York: Holt, Rinehart, and Winston, Inc., 1960), p. 141.

A stock solution of o.4% sodium hydroxide (NaOH) is provided for your use. An 0.04% solution can be made from the stock by adding 90 ml of distilled water to 10 ml of stock solution. Each ml of this 0.04 solution will combine with 10 micromoles of carbon dioxide. A micromole is a chemical quantity that contains 6×10^{17} molecules (600,000,000,000,000,000).

A solution of phenolphthalein is also provided. This is a dye which turns red after all the carbon dioxide has been removed from a sample of water.

To gain some experience in measuring carbon dioxide content of water, carry out the following experiment:

Measure 100 ml of tap water with a graduated cylinder and transfer it to a bottle or Erlenmeyer flask. Add 3 to 5 drops of phenolphthalein. Then bubble air from your lungs through the water, using a glass tube or a soda straw, for about one minute. Now slowly add 0.04% NaOH, using a graduated pipette or burette, and mixing repeatedly by swirling the water around to insure complete reaction between NaOH and CO_2. When the solution turns pink, record the number of ml of 0.04% NaOH used. To compute the number of micromoles of CO_2 in the water sample, simply multiply the ml of NaOH used by 10.

Repeat this exercise at least twice to give you experience in approaching the end point (color change) carefully, so that your determination of CO_2 content will be as accurate as possible.

Information for the Teacher

Activity 1. Physical and biological factors of the environment should be considered.

Activity 2. Determination of pH can be made easily with test paper. Supply houses sell pH test paper of all ranges. The Marshalltown science department has a pH meter

which will be used in addition to the test paper.

There are kits available that simplify the tests for pH, oxygen, carbon dioxide, and other water-soluble materials. The kits can be used in the field quickly and easily.

The Hach Chemical Company in Ames, Iowa manufactures separate kits and combination kits for testing more than twenty water soluble substances including detergents.

Planaria are sensitive to environmental conditions. Therefore, it would be possible to guide a student to study the effect of fluctuating external influences on planaria. Planaria live in a pH range of 6-9.[1]

EXERCISE IV. THE USE OF A KEY

Directions for the Student

In order to know planaria better, secure training in careful observation and in the use of a dichotomous key. It is desirable to identify the specimens obtained in the field or furnished by the instructor.

The phylum Platyhelminthes includes three classes. The classes Cestoidea (tapeworms) and Trematoda (flukes) are entirely parasitic. The class Turbellaria is almost exclusively free-living with externally ciliated bodies.

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[1]Stanley Shaw and D. D. Copeland, "The Use of Planaria in High School Laboratories," The Welch Biology and General Science Digest, XII (No. 2, 1963), p. 5.

The Turbellaria are usually less than 25 mm in length and some are microscopic. Some are fresh water, others marine, and still others are terrestrial forms. Most turbellarians have flattened, elongated bodies, though some are cylindrical in form. External characteristics usually noted include eyes, sensory pits, and bristles.

All turbellarians, except the sub-class Acoela, have a single cavity which may or may not be branched. One opening functions as a digestive tube or aperture into the cavity and serves as both mouth and anus. There is a single genital pore behind the mouth. The mouth is ventral and located on the mid-ventral line anywhere from the middle of the body to the anterior end. The mouth leads directly to a muscular pharynx which is housed in a pharyngeal cavity. In most turbellarians the pharynx can be protruded through the mouth by muscular elongation, so that it projects freely downward or posteriorly. Turbellarians are mostly carnivorous taking in food through the extended pharynx.

Fresh water turbellarians are hermaphroditic and reproduction is usually sexual, involving copulation and mutual exchange of sperm. However, asexual reproduction by transverse fission or fragmentation is not uncommon. The reproductive system is very complex in the Turbellaria and can be studied only by serial sections. Generic and

specific identification of the Turbellaria is based on details of the reproductive system and is very difficult. Specialists have produced workable keys using in part external characteristics such as head shape, size, color of body and eye arrangement.

The dichotomous (two choice) key presented below is adapted from Pratt's Manual of the Common Invertebrate Animals, 1951, and Ward and Whipple's Fresh-Water Biology, 1959.

> As scientists identify and classify the myriads of organisms, living and extinct, it becomes increasingly important that devices are developed which may aid others in recognizing these organisms. A key is this kind of device, and its use may aid the recognition of organisms. Only a tentative identification can be made with the aid of a key. Descriptions in textbooks, monographs or in some cases the original description of an organism is essential before one can be relatively sure of the identity of a species in question. Even after delving into original sources one may be unable to make a positive identification. Experts in a field often disagree upon names assigned specific organisms or upon the organisms associated with a given name.[1]

In using the key, start at number 1. Choose the one of the paired statements which is applicable to the specimen. If identity is not established by this choice, proceed to the numbered statement indicated at the end of the line. Continue this procedure until identity is established. The number in parenthesis in the second column indicates the

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[1]Leland P. Johnson, "A Key to Some Fresh Water Protozoa," Bios, Beta Beta Beta, 1956, p. 3.

number of the previous paired statement which leads to this line. This allows the key to be worked backwards if an organism but not the identifying characteristics is known.

For a more extensive account of the classification of flatworms, see Ward and Whipple[1], Pratt[2], and Pennak[3].

Key to Orders of Turbellaria

1a Minute marine forms without intestine...Subclass Acoelata (Not treated here)

1b Intestine present... Subclass Coelata 2

2a (1) Small forms with a straight intestine...Order (Not treated here--recently split into 3 orders) Rhabdocoelida

2b Usually larger forms with branched intestine... 3

3a (2) Intestine with many large branches...Order Polycladia (Not treated here)

3b Intestine with 3 main branches, 1 branch extending anterior to the head, the other 2 posteriorly... Order Tricladida

The order Tricladida is the best known of the free-living Turbellaria. Triclads, as they are called, have heads which are more or less obvious varying in shape from

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[1]Henry Baldwin Ward and George Chandler Whipple, Fresh Water Biology, (New York: John Wiley and Sons, Inc., 1959), pp. 325-334.

[2]Henry Sherring Pratt, Manual of the Common Invertebrates, (Philadelphia: The Blakiston Co., 1951). pp. 170-190.

[3]Robert Pennak, Fresh-Water Invertebrates of the United States, (New York: The Ronald Press, 1953), pp. 114-140.

triangular to truncate with auricles, ear-like projections, more apparent when the head is triangular. Two eyes are usually present (cave dwelling species have no eyes) although a few species have more than one pair of eyes. Colors vary from white to black with shades of gray and brown the most common colors.

Key to Species of Order Tricladida

1a Aquatic triclads 2

1b Terrestrial triclads... suborder Terricola
(Not treated here)

2a (1) Fresh-water triclads; planarians suborder Paludicola 3

2b Marine triclads... suborder Maricola
(Not treated here)

3a (2) Inner muscular zone of the pharynx with distinct circular and longitudinal layers... 4

3b Inner wall of pharynx with several alternating circular and longitudinal muscle layers... family Dendrocoelidae 21

In N.A. most colored planarians and all planarians with triangular heads belong to the Planariidae. The family to which white planarians belong (all white planarians in N.A. have truncate heads) cannot be determined without examining cross sections through the pharynx.

4a (3) Without an adhesive organ in the center of the anterior margin; nearly always with eyes; colored or white.. family Planariidae 5

4b With an adhesive organ; white, eyeless cave dwellers... family Kenkiidae
(Not treated here)

5a (4) Eyes 2 (sometimes wanting in Phagocata subterranea) accessory eyes sometimes present as abnormalities... 6

5b Eyes numerous... 19

6a (5) Head triangular; colored... 7

6b Head truncate or nearly so; colored or white... 11

7a (6) Head very triangular, with prominent auricles; testes numerous, extending the body length... genus Dugesia 8

7b Head of low triangular form, with low auricles; testes few, prepharyngeal... 10

8a (7) Auricles narrow, pointed; ovovitelline ducts entering the bursal canal separately...Dugesia dorotocephala (Woodworth)

Largest N.A. planarian, to 25 mm or more, uniformly dark brown to black, sometimes with a light mid-dorsal stripe. Pennsylvania and Virginia, west to the Pacific coast; usually in springs or spring-fed waters. Includes agilis.

8b Auricles broad, blunt; ovovitelline ducts unite at entrance into bursal canal... 9

9a (8) Copulatory sac large... D. tigrina (Girard)

Moderate size, 15-18 mm long, often smaller, color very variable, most commonly spotted brown and white or brown with a wide white mid-dorsal streak; most common N.A. triclad. Everywhere in ponds, lakes, rivers, on vegetation, under stones. Old name, Planaria maculata.

9b Copulatory sac small... D. Microbursalis Hyman (1931)

Small, dark, almost black, 10-12 mm long, shape same as D. tigrina.

10a (7) Penis normal with bulb and pipilla...Cura foremanii (Girard)

Short, broad, plump, to 15 mm long; colored uniformly seal brown or dark gray to black; auricles

with a slanting white dash (auricular sense organ); capsules spherical, stalked; will lay capsules continuously in well-fed laboratory cultures. New England and Canada, west into Michigan, south into Tennessee and North Carolina; in cool creeks and rivers. Old Name, Planaria simplicissima.

10b Penis degenerate without bulb and with very small papilla... Hymanella retenuova Castle (1941)

Small to 14 mm long but usually less; grayish; capsule oval, not stalked, retained for a long time in the male antrum. Massachusetts, Deleware, North Carolina; vernal ponds and spring fed swamps.

11a (6) Copulatory complex with an adenodactyl; colored... Planaria dactyligera Kenk (1935)

Small slender, to 13 mm long; uniformly dark brown or gray to almost black; cannot be distinguished from species of Phagocata except by the adenodactyl, observable only in sections, capsules spherical or slightly oval, not stalked. Virginia; springs, spring-fed swamps and ponds.

11b Copulatory complex without an adenodactyl; colored or white; capsules always spherical, not stalked... Phagocata Leidy 12

12a (11) Polypharyngeal... 13

12b Monopharyngeal... 15

13a (12) Colored... 14

13b White... Phagocata subterranea Hyman (1937)

Small, about 5 mm in length, sometimes eyeless. Caves; Indiana.

14a (13) Penis papilla long and pointed...P. gracilis gracilis (Haldeman)

Relatively large, broad, 15-20 mm long; margins of head expanded; uniformly colored dark gray to brownish or grayish black. Pennsylvania and Virginia, mostly in springs; common.

14b Penis papilla truncate...P. gracilis woodworthi Hyman (1937)

Cannot be distinguished from P. gracilis gracilis except by details of the copulatory apparatus. New England went to the Delaware River.

15a (12) Colored... 17

15b White... 16

16a (15) Testes extending to posterior end...P. nivea Kenk (1953)

Small, delicate, to 8 mm Alaska only.

16b Testes extending to level of mouth...P. morgani (Stevens and Boring)

Larger, 10-17 mm. Springs and creeks throughout the Appalachian region, also Canada, Wisconsin, Michigan. Old name Planaria truncata. 19b

17a (15) Ejaculatory duct with ventral blind sac...P. velata (Stringer)

17b Ejaculatory duct without ventral blind sac... 18

18a (17) Copulatory sac sacciform...P. gracilis monopharyngea Hyman (1945)

Externally indistinguishable from the other subspecies of P. gracilis but monopharyngeal; 15 mm. or more. Iowa, in a drain.

18b Copulatory sac U-shaped... P. vernalis Kenk (1944)

Indistinguishable from P. velata except by details of the copulatory apparatus; also has habit of fragmentation and encystment. North Central states; temporary ponds, winter and spring. Seldom sexual.

19a (5) Eyes in a band around anteriour end...Polycelis Enrenberg 20

19b Eyes in 2 groups in usual site; white...Phagocata morgani polycelis Kenk (1935)

Identical with P. morgani except for the eyes. Virginia; springs and creeks. See 16b

20a (19) Penis papilla short, truncate; sperm ducts entering penis bulb asymetrically... Polycelis coronata (Girard)

Large, 15-20 mm. long, uniformly dark brown or black, head rounded with projecting auricles. Hill

and mountain streams; Black Hills of South Dakota, westward to the Pacific Coast.

20b Penis papillae elongate, pointed; sperm ducts asymetrical... Polycelis borealis Kenk (1953).

21a (3) Eyes in 2 longitudinal groups in usual position... Sorocelis americana Hyman (1937)

Each eye group with 6 to 20 eyes; white, with small adhesive organ; slender, 12-15 mm. long. Ozark region; caves, also springs, outside of caves. Very abundant when present.

21b Eyes otherwise or absent... 22

22a (20) White, with or without adhesive organ... 24

22b Colored, with adhesive organ, eyes 2... 23

23a (22) Penis papilla wanting...Rectocephala exotica Hyman (1954).

Black, 14 mm. long; bursal canal very long. Known only from lily pond, Washington, D.C.; possibly imported.

23b With long pointed penis papilla...Dendrocoelopsis piriformis Kenk (1953).

Brown or brownish gray, may be striped; bursal canal of usual length. Alaska; streams.

24a (22) With normal male copulatory apparatus... 26

24b With massive penis bulb and reduced penis papilla; ejaculatory duct runs ventrally in penis bulb... Procotyla Leidy 25

25a (24) Adhesive organ present; eyes irregular in usual sites... Procotyla fluviatilis Leidy

Large, broad, thin, to 20mm. long. New England, west to Wisconsin and Illinois, also Canada, south to North Carolina; most common white planarian of the eastern U.S.; springs, ponds, streams, lakes.

25b Without adhesive organ; eyes 2 or wanting...P. typhlops Kenk (1935)

Small, slender, to 12 mm. long; rare. Springs, subterranean habitats; Virginia, Florida.

26a (24) Adhesive organ evident; white...

26b Adhesive organ only microscopically determinable; penis papilla very short...Dendrocoelopsis alaskensis Kenk (1953).

Streams; Alaska.

27a (26) Without eyes; penis bulb surrounded by large eosinophilous mass...Macrocotyla glandulosa Hyman (1956).

Stream below cave; Missouri.

27b With 2 eyes; penis bulb not with eosinophilous mass... Dendrocoelopsis vaginatus Hyman (1935).

Large, 15-20 mm. long. Flathead Lake, Montana.

EXERCISE V. MORPHOLOGY

Directions for the Student

Activity 1. The student should have learned that positive, specific identification of planaria necessitates the study of serial sections through the pharynx and copulatory structures in sexually mature specimens. It is also necessary to observe serial sections of planaria when studying morphology. Students will prepare their own slides, using the paraffin method of preparing the material for sectioning.

Sagittal, frontal, and transverse sections should be prepared.[1] These sections are described as:

A. Sagittal. A section that follows the mid-dorsal line and runs the entire length of the body, dividing

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[1]Peter Gray, Handbook of Basic Microtechnique, Second Edition, (New York: McGraw-Hill Book Co., Inc., 1958), p. 124.

the body into more or less equal right and left halves.

B. Frontal. A section parallel with the long axis of the body and at right angles to a sagittal section. It divides the body into a dorsal and ventral part.

C. Transverse. A section perpendicular to the long axis of the body. It divides the body into an anterior and a posterior part.

Students may work individually, in pairs, or in groups of three.

A method for preparing slides from Feldman's Techniques And Investigations In The Life Sciences, is shown below. It is a discussion, as well as giving instructions, of biological microscopic techniques. One fixative and one stain were selected to illustrate the procedures which are discussed. Where helpful, notes applying to planaria may be inserted.

PARAFFIN SECTIONS[1]

Tissues must undergo a number of different steps before they can be sliced or sectioned for slides. The following sequence of procedures is the usual way of successfully preparing microscopic slides: 1) Fixation; 2) Washing; 3) Dehydration; 4) Clearing; 5) Embedding in paraffin or Infiltration; 6) Sectioning; 7) Affixing of Sections; 8) Staining; 9) Mounting.

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[1]Solomon Feldman, Techniques and Investigations in The Life Sciences, (New York: Holt, Rinehart and Winston Inc., 1962), pp. 109-115.

Fixation. A piece of tissue removed from a living organism soon ceases to retain its lifelike characteristics because of bacterial decomposition, breakdown of cellular proteins by enzymatic action, and/or shrinkage due to loss of water and dissolved materials. In order to retain the lifelike characteristics of the tissue as much as possible, we place it in a liquid called a fixative. This solution preserves and hardens the tissue.

This procedure minimizes the effects of subsequent treatment with alcohols, paraffin, and of sectioning. Fixation also causes the proteins to precipitate in a form similar to that found in the living state. The fixative prepares the tissue for a specific staining reaction. It accomplishes this by combining with the dye and the tissue. Differences in optical properties (refractive index) of the tissue components are enhanced by proper fixation, therefore making their resolution much easier.

The fixing solutions described below are but a few of the many commonly used in laboratories today. Since we want the tissue to be as lifelike as possible, use only living or freshly killed biological material for fixation. Use a volume of fixative 40-60 times the volume of tissue.

Small pieces of tissue (10x10x5 mm) are best for fixation. Place the specimen on a cork and slice it with a sharp single-edged safety razor blade. Keep the tissue moist with physiological saline solution. The solution used with tissues of coldblooded animals and invertebrates consists of 7.5 grams of sodium chloride in one liter of distilled water. Use 9.0 grams for tissues of warm-blooded animals.

[Place living planaria in a watch glass containing a little water. Flood specimen with 2% nitric acid or 2% magnesium sulfate. The percentage of nitric acid or magnesium sulfate may need to be varied. After movement ceases, transfer worms to fixative.]

After slicing the tissue, immerse it in wide mouth bottles with cork stoppers and label appropriately. It is best to prepare containers of fixatives ahead of time so that there will be no delay in fixation.

Composition of Fixing Solutions

. . .

D. Bouin's solution

Picric acid (saturated aqueous solution, 1 gram/75 ml distilled water)	75 ml
Formalin	25 ml
Glacial acetic acid	5 ml

Time--12-48 hours

Bouin's fluid is one of the safest fixatives a beginner can use. It is almost foolproof. It gives excellent results and can be used for most tissues.

After fixations wash in 70% ethanol until the yellow color is removed. Residual picric acid crystals can be removed before staining by immersing sections in 70% ethanol saturated with Li_2CO_3.

Washing. After the tissue has been fixed, it is thoroughly washed with a suitable medium. In general, tissues fixed in solutions containing alcohol are washed with alcohol of the same concentration as is contained in the fixative. Tissues fixed with aqueous solutions are washed in running water. Bouin's fixative or other solutions containing picric acid are washed with alcohol. Complete removal of the fixative is essential for proper staining of the tissues.

Dehydration. Water does not mix with paraffin nor with many of the reagents used to prepare permanent microscopic slides of plant and animal tissues. Therefore, tissues must be dehydrated prior to infiltration with paraffin.

We do this by passing the tissue through a series of graded ethyl alcohols of increasing concentrations which gradually replace the water in it. An abrupt transfer of the tissue from water to a solution containing a high concentration of alcohol produces strong diffusion currents which cause distortion of the biological material. Dehydration must therefore be a gradual process.

In general, water is removed from tissues by passing them through a series of alcohols of increasing concentration, such as, 30%, 50%, 70%, 85%, 95% and two changes of 100% ethyl alcohol. For cell studies the concentration of alcohols must be even more gradual.

The average piece of tissue is permeated with each alcohol in about 20 to 30 minutes. Larger pieces of course, require a longer stay in each concentration of alcohol. Tissues can be stored in 70%-80% alcohol.

Besides ethyl alcohol, acetone, isopropanol and butanol may be used to dehydrate tissues.

Clearing. Paraffin is almost completely insoluble in alcohol, therefore the alcohol must now be replaced by a substance in which both paraffin and alcohol are soluble. This process is called clearing the tissue. A number of substances such as xylol, toluol, and n-butanol mix with both alcohol and paraffin.

Besides preparing the tissue for infiltration with paraffin, the clearing agent also makes the tissue more transparent.

Infiltration. The material is now ready for infiltration, i.e., replacing the clearing agent with liquid paraffin. To achieve this, a heating device for maintaining paraffin in a liquid state is needed. In its simplest form, such a device consists of an incandescent bulb set in a shade or reflector suspended above a vessel containing paraffin. The height of the lamp is adjusted to provide sufficient heat to melt the upper part of the paraffin to a depth of 1 to 1½ inches, while an equal thickness of the paraffin remains solidified. When the tissues are placed in the paraffin they will lie at the point where the paraffin is melting.

Use small metal cups in which to melt paraffin and infiltrate the tissue. . . . Adjust the height of the lamp so that about one half of the paraffin melts in the cups and the other half remains solid. This prevents overheating of the tissue.

Paraffin which melts at 50° to 55° C is used for infiltration and embedding of the tissues. The cleared tissue is transferred to a metal cup containing equal volumes of liquid paraffin and the clearing fluid. After one half hour, the tissue is again transferred to pure molten paraffin for two hours. The paraffin should be changed at least once during this time. As a result of this treatment, the clearing agent is removed and the tissue is infiltrated with liquid paraffin.

Embedding. After the tissue has been infiltrated with paraffin, it is ready to be cast into a mold. Once in the mold, the tissue will be ready for sectioning into thin slices. Small pieces of tissue may be embedded in capsules, such as those used to pack drugs. They come in assorted sizes. Use a capsule at least twice the size of the block of tissue. Supports for the capsules are made by drilling holes of appropriate size in a block of wood. Make the holes deep enough to set the capsule in half way.

Place the empty capsules in their supports. Use a warm medicine dropper to pour enough melted paraffin to cover the bottom of the capsule. With a warm forceps, transfer the tissue to the capsule. Now fill the capsule with melted paraffin. Orient the tissue in the paraffin so that it lies perpendicularly to the plane of the knife. Place the first part to be sectioned uppermost. Pass a heated needle through the paraffin around the embedded material to remove air bubbles which may be present. As soon as a thin surface film forms on the paraffin, immerse the capsule in cold water. This prevents the formation of crystals and cools the block rapidly. When the capsule is solid remove it from the water.

Identify the material in the capsule by affixing a paper label to the outer surface of the capsule with melted paraffin. Such identified capsules can be stored for years.

Specimens can also be embedded in a variety of small glass or plastic dishes. The inner surface of the dish should be coated with a thin layer of glycerol before the tissue is embedded. This facilitates the removal of the solidified paraffin block. The block is then trimmed to size for sectioning. Leave enough paraffin to mount the block for sectioning.

Sectioning. In general, tissues must be reduced in size in order that light may pass through them when they are examined with a light microscope. The common practice is to cut extremely thin slices about 10-15μ thick, (1μ=1 micron=1/1000 mm.). Of course, tissues that are already very thin, thin whole specimens, or specimens which are squashed or smeared need not be cut.

It is possible to obtain accurately cut slices of uniform thickness with a microtome. However, this instrument is expensive and not readily available except in biological laboratories. Sections can also be cut freehand or by a simple, inexpensive well or hand microtome.

Free-hand sectioning. Although this method may not produce uniformly cut sections for microscopic study, with some practice, it is possible to produce usable sections for microscopic preparations.

It is best to use a barber's straight razor or a safety razor blade to cut freehand sections from fixed tissue and paraffin-embedded tissue. To do so, support both forearms against a table's edge. Hold a piece of fixed tissue with the thumb and index finger of the left hand. Grasp a single-edged, safety razor with the thumb and index finger of the other hand. Place the razor blade flat on the embedded tissue, and with your index finger on the back of the blade as the force, carefully push the razor through the embedded tissue. In this manner you can slice off a thin, uniform section. Keep the tissue and the blade wet as this facilitates sectioning and reduces curling of the section during cutting.

Tissues which are not embedded in paraffin are supported by recessing them between pieces of pith or carrot. Cutting is done through the supporting material and the tissue, keeping the razor blade at right angles to the tissue.

Sectioning with a hand microtome. Essentially, a microtome consists of some device which will move a block of tissue through a given distance in order that a stationary blade may slice off a piece of tissue of uniform thickness. The converse is also possible.

There are a number of simple, inexpensive instruments called hand-well microtomes with which it is possible to cut sections of a determined thickness accurately. Such devices consist of a cylinder which has a disc-shaped metal plate threaded to its upper end. A glass surface is attached to this plate, which serves as a guide for the razor. A recessed well is moved up or down by a micrometer screw. Each mark on the micrometer equals 10μ or 1/100 mm. The object to be sectioned is placed in the well and fastened within the well by a set screw.

When cutting sections, hold the microtome firmly with one hand and draw the razor blade through the tissue with the other. Make certain that the razor is always flat and in contact with the glass surface, thus insuring a section of uniform thickness.

Affixing Sections. After the tissue is sliced, transfer each section from the blade to a dish of luke-warm water by using a fine, camel's hair brush.

Sections to be stained are usually mounted on slides. They must be firmly attached to preclude their falling off during continued processing. Mayer's albumen adhesive is usually used to affix paraffin sections to slides. Prepare this solution by mixing 50 ml of glycerol with 50 ml of filtered egg white. Add 1 gram of sodium salicylate or several crystals of thymol to inhibit growth of fungi.

Coat slides with the adhesive by placing a minute drop on a clean slide and then rubbing it with a finger, leaving room at the side for a slide label. Apply only enough adhesive to barely coat the slide. Now place a drop of warm distilled water on the slide and lay the cut paraffin sections on the water. Gently warm the slide by holding it over a lighted bulb until the paraffin flattens out and is free of wrinkles. Drain off the excess water and put the slides away to dry. Make certain that the slides are properly marked with a wax crayon or a graphite marking pencil. Cover all slides with wax paper to prevent accumulation of dust or other materials.

Pre-staining Processing. Most stains are dissolved in distilled water, and some are soluble in alcohol.

Paraffin does not mix with either of these liquids and therefore must be removed from the sections. This is achieved by immersing the slides in xylol which dissolves the paraffin.

Xylol is practically insoluble in water or dilute alcohol, therefore all traces of it must be removed from the sections before continuing their processing. This is accomplished by placing the slides in absolute alcohol which is miscible with both water and xylol.

If the sections are to be stained in water-soluble dyes, they should be passed through a series of alcohols of decreasing concentration (95%, 80%, 70%, 50%, 35%) and then into distilled water. Of course if an alcohol-soluble stain is used, the section is only processed through 95% alcohol and then stained.

Staining. In unstained tissue, it is possible to distinguish various components only if they have different refractive indices. These differences can be more readily detected by proper staining methods, i.e., the coloring of biological material with dyes. Cells, cell structures and tissues react differently to similar stains because of inherent chemical and physical differences. Consequently, these components become more easily defined.

Specimens must be stained enough to show the components of the tissues. If understained, the structures are not readily discernible. If overstained, the tissues may be too dense to make identification possible. Successful microscopic preparations can be achieved by overstaining the section, then destaining until the proper intensity is obtained. Destaining or differentiation is conducted with a microscope. The slide is placed in a petri dish containing the differentiating solution and examined microscopically.

In general, basic dyes such as hematoxylin and safranin are differentiated by a weak acidic solution. Eosin or light green which are acid stains can be differentiated by an alkaline solution consisting of 0.1% ammonium hydroxide in 95% alcohol. Differentiation must be slow enough so that it can be stopped when the microscopic section is placed in the next alcohol of a dehydration series.

Counter-Staining. In this procedure, sections already stained with a nuclear stain are treated with one or more additional stains which bring out the contrast between the different cells and the tissue elements.

Preparation of Solutions.

1. Prepare dilutions of ethanol from 95% ethanol rather than the expensive absolute alcohol. Since dilutions in the graded series of alcohols need not be critical, dilute solutions of ethanol can be prepared the following way. Use 95% ethanol as a base. To convert to any percentage of alcohol, merely use the percentage of alcohol as the number of ml of 95% alcohol to use and then add the difference in distilled water.

For example, 80% ethanol is prepared by combining 80 ml of ethanol (80 ml for the 80%) and 15 ml of distilled water (95-80=15), to the 80 ml of 95% ethanol.

2. Isopropanol may be substituted for ethanol in dehydration and hydration.

Staining Solutions and Procedures.

As in the other phases of biological techniques, experience is the teacher, rather than written directions. The length of time required for the staining of tissues varies with the technique, stain, fixative and the tissue. The following procedures suggest general methods for preparing stained microscopic slides.

The first of these is described in great detail. This information will serve as a guide when using the more briefly outlined staining methods which will follow it.

A. Ehrlich's Acid Hematoxylin. Best results are obtained when tissues are fixed in Bouin's or Zenker's fixatives when using this stain. This stain is recommended as a general tissue stain.

Composition of Stain

Hematoxylin	0.67 g
Absolute alcohol	32.0 ml
Glacial acetic acid	3.0 ml
Glycerol	32.0 ml
Potassium alum	3.0 g
Hot distilled water	32.0 ml

Dissolve the hematoxylin in alcohol, then add the acetic acid and the glycerol. Dissolve the alum in the hot water and add it to the hematoxylin solution while stirring. Cover the bottle with a gauze pad and let it age for several weeks. When the solution acquires a deep red color, it is ready for use. When stored in closed bottles it keeps for months.

Treatment Before Staining. Remove Paraffin from the mounted sections by immersing them in xylol for 1 to 5 minutes. Transfer the slide to another change of xylol for 1 to 3 minutes.

Place the slides in a mixture of equal volumes of xylol and absolute ethanol for 5 minutes.

Remove the xylol from the tissue by immersing it in 2 changes of absolute alcohol.

Bring the sections to distilled water by running them through 95, 70, 50, and 30 per cent ethanol and then to water. Leave the sections in each solution for at least 2 minutes.

Staining. Transfer the mounted sections to the staining solution. Stain them for 1 to 10 minutes.

Differentiation. Rinse the slide in distilled water and place it in a petri dish containing tap water. Examine the sections microscopically. In alkaline tap water the nuclei appear blue (blueing process) while the cytoplasm remains colorless. If the sections are understained, i.e., the nuclei are not distinct and blue, rinse the mounted section in distilled water and repeat the staining.

If the nuclei are dark and not discernible, dip the sections in dilute HCl for several seconds, rinse them in distilled water and examine in tap water. Repeat the treatment if necessary.

Wash the sections in running water for about 60 minutes.

Dehydration. Remove the water from the sections by immersing the slides for at least 2 minutes, in each of 30, 50, and 70% ethanol.

Counterstaining. Eosin Y stains cytoplasmic structures. Consequently, it is used as a counterstain with hematoxylin. It is substituted by Erythrosin in plant tissues.

Dissolve 0.2 gram of eosin Y in 100 ml of 95% ethanol. Counterstain in this solution for 10-30 seconds. Remove the slides and rinse them in 2 changes of 95% ethanol for 5-10 seconds in each. Transfer the slides to 100% ethanol.

Examine the slides microscopically. The cytoplasm of the cells should appear pink (do not let the sections dry out). If the stain is too light, return the slide to 95% ethanol and restain. If the stain is too heavy, return the slides to 95% ethanol to destain. Examine the sections microscopically. If the stain is now a light pink, it is satisfactory. Dehydrate in absolute ethanol for 5-10 minutes.

Transfer the slides through a solution consisting of equal volumes of xylol and ethanol.

Process the slides through 2 changes of xylol, of 3 minutes each. If the clearing agent (xylol) is cloudy, it indicates that all the water has not been removed from the tissues. Return the slides to the alcohols for continued dehydration.

Mounting the Cover Glass. After the sections are stained, dehydrated, and cleared, they are covered with a protective medium and a glass cover (cover slip or cover glass). This makes the microscopic preparation a permanent one.

To make a permanent mount, grasp one edge of the cover slip with a forceps. Rest the opposite edge on the slide in contact with the drop of mounting medium. The cover glass should make a 45° angle with the slide. Let the mounting medium flow along with the cover slip as you

lower it slowly on the slide, allowing the medium to advance, forcing the air out. Air bubbles which may get caught can be removed by slight pressure on the cover glass with a needle or other object. Remove any excess of the mounting medium with a paper towel or soft cloth, and set the slides aside on a flat surface to harden.

The most commonly used mounting media are Canada balsam and gum damar both of which are soluble in xylol. These are usually used after alcohol dehydration. Permount is a synthetic medium also used after alcohol dehydration.

Sections can be mounted in euparal directly from 95% ethanol. Its advantage is its low index of refraction, so that faintly stained materials indiscernible in balsam are made visible. With the hematoxylin stains, use the green euparal. To thin euparal, a special solvent called "euparal essence" is needed.

After removing the excess mounting media, identify the sections by affixing a label to one end of the slide. On it, indicate the type of tissue or organ, the fixative, the stain, and the date.

Activity 2. Use the microscope to observe the slides you have prepared. Make detailed drawings of observed sections, labeling structures and tissues, and identify the serial section that was observed.

Information for the Teacher

Activity 1. Prepared slides may be purchased if desired to use for comparing with the student's work. Insufficient time may necessitate using purchased slides.

Activity 2. Gross structures will be easily identified. If sections of regenerating areas are prepared, guide

the students' attention to the neoblastic cells. Neoblastic cells are distributed throughout planaria's body but when an injury occurs the neoblastic cells migrate to the point of injury. The neoblastic cells have the capacity to differentiate and begin the reforming of the missing or injured part.

It might be suggested that sections be prepared while studying regeneration.

EXERCISE VI. REGENERATION

Directions for the Student

Activity 1. Many living things have the ability to grow or regenerate missing body parts. Some lizards, when grasped by a predator, can snap off the tail which continues to wiggle. While the predator is occupied with the tail, the lizard attempts to escape. If the escape is successful, the lizard regenerates a complete tail. Among the invertebrates, regeneration is common, and some can regenerate complete organisms from small pieces. The repair or healing of a wound or broken bone is regeneration, and the development of a whole plant from a cutting or leaf slip may also be considered regeneration.

Many organisms are suitable for studying regeneration,

Salamanders grow new limbs, Planaria and Hydra grow complete organisms from fairly small pieces and tadpoles can grow new tails. However, as a general rule, capacity to regenerate lost or injured parts becomes less as the organisms become more complex.

Directions for three simple operations are given below.

Directions:

> Type 1 Operation. Prepare three labeled dishes of water, one for anterior pieces, one for middle pieces, one for posterior pieces. Transfer a planarian from the stock dish to a slide or cork, using the brush to pick up the animal. Wait until the animal extends itself in crawling and then cut off the head. . .Next, cut off the posterior end. The pharynx is usually extruded and lost during the operation, and should be discarded. Place the pieces in the appropriate dishes. The experiment should replicated several times, keeping all similar pieces in one dish.
>
> Type 2 Operation. Follow the directions for a Type 1 operation, except make the cuts obliquely (at a slant). . .
>
> Type 3 Operation. Using approximately the same technique as for Type 1, make a single longitudinal cut, separating the right half of the animal from the left half. . .[1]

Activity 2. The student will devise three additional operations: (1) to produce a planaria with two heads, (2) to experimentally alter the anteroposterior gradient by cutting a piece that will produce a head at each end of the piece or

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[1]Don E. Meyer and Virginia M. Dryden, Biological Science An Inquiry Into Life, Student Laboratory Guide, (Chicago: Harcourt, Brace and World, 1963), pp. 209-210.

a tail at each end of a piece,[1] and (3) one of the student's choice.

Make a report including drawings of regeneration occurring for each of the six operations.

The care of operated pieces is the same for all types of operations. Dishes, clearly labeled, should be covered and kept in a cool place with dim light. High temperatures are one of the principal causes of mortality. Do not attempt to feed regenerating animals. Daily observation is necessary to remove any dead pieces. If death of a part has occurred, change the water in that dish as the products of decomposition are harmful to living pieces. The water in all dishes should be changed every third day.

Optional Activity

The following is an abstract of "Electrical Control of Polarity in Regenerating Dugesia tigrina". The student with the information given may attempt to duplicate the research.

ABSTRACT

Cut pieces of D. tigrina of known original polarity, one to ten mm. "relaxed length," were imbedded in 3% agar and exposed to direct current at room temperature for (usually) five days. Aerated 1/20 Ringer's solution

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[1]Ralph Buchsbaum, Animals Without Backbone, (Chicago: University of Chicago Press, 1948), pp. 128-129.

in tap water flowed through the wedge trough to provide oxygen and eliminate electrode products.

Pieces oriented with anterior end to cathode regenerated normally at all current densities. Pieces oriented anterior end to anode: (a) at or below 5 μa./mm regenerated normally; (b) between 6 and 14 showed increasing head behavior in cathode end (tail), becoming subsequently normal; (c) at 15-17 developed head structure and functions in cathode end with subsequent reversion to normal polarity; (d) at 18 became permanently two-headed with locomotor competition, common intestine and pharynx usually at right angles to axis; (e) at 19 became bipolar with subsequent suppression of anode head; (f) at 20 and above underwent complete reversal of polarity with head behavior frequently persisting temporarily in tail (original anterior end). Current densities are approximate averages. Length of piece and original body position were not related to effect of current. The potential gradient at reversing strength was about 200 millivolts per millimeter. No "growth inhibition" was found at densities up to 31 μa./mm. Mortality was high, due principally to motor activity of pieces preventing healing, opening lesions and, possibly, anoxia.[1]

Information for the Teacher

Activity 1. Regeneration buds appear usually within two or three days. The cells in the bud are unpigmented. Anterior ends of cut pieces will regenerate heads, and posterior ends will regenerate tails with regeneration occurring faster at the anterior end. A new head complete with eyes will regenerate in fifteen days on the middle piece.

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[1]Gordon Marsh and Harold W. Beams, (State University of Iowa), "Electrical Control of Growth Polarity in Regenerating Dugesia Tigrina," Federation Proceedings. VI, No. 1, 1947, 163-164.

In operation two, the initial regeneration bud is approximately at a right angle to the cut but soon straightens out so that the resulting animal is not crooked.

Occasionally regenerated parts are irregular in shape or pigment.

Activity 2. A planaria with two heads can be produced by cutting the anterior end down the middle, stopping short of the pharynx. The incision must be kept open by repeated cutting to prevent healing of the wound.

The anteroposterior gradient is so named because a piece of a planaria retains the polarity it had while a part of the complete animal. This means that a regenerated head grows from the portion of the piece which faced the anterior end of the whole animal and a regenerated tail from the cut end which faced the posterior end.

If a planaria is cut transversly just behind or through the auricles, a second head facing the posterior direction is produced. Some investigators report that a narrow piece cut from the posterior region of a planaria will regenerate a tail at both ends.

EXERCISE VII. RESPIRATION

Directions for the Student

Activity 1. In a previous exercise you learned to

measure the carbon dioxide content of water. You can use the same method for measuring respiration by aquatic organisms, and to compute it as micromoles of CO_2 given off per gram of organism per hour.

MEASURING THE RESPIRATION RATE OF AQUATIC ORGANISMS[1]

PROCEDURE: Use two bottles, each of about 140-ml capacity. Label the bottles A and B. Place in bottle A an aquatic organism that will fit in the bottle comfortably, or if the organism you select is small (germinating seeds, aquatic plants, insect larvae, snails, etc.) collect 10 or more individuals and put them in bottle A. Draw about 500 ml of tap water and add 5 drops of phenolphthalein. If the water turns pink, carefully bubble respired air through it until it just turns clear. Fill bottles A and B with this water (B should not contain any animals). Note the time of beginning the test.

OBSERVATIONS AND COMPUTATIONS: After about 30 minutes, determine the CO_2 content of all the water in each bottle, by using the method described . . . (on page 45). After weighing the organism or organisms you used, compute the respiration rate as follows:

$$\frac{\text{Difference between micromoles } CO_2 \text{ in A and B}}{\text{Weight of animals in A x hours of test}} =$$

Rate of respiration in micromoles CO_2 per gram per hour

For example:

If bottle A had 32 micromoles of CO_2 in it, and bottle B had 11, and if A contained 7.3 grams of snails, and the test was 35 minutes long (0.58 hr.), then the respiration rate would be computed as follows:

$$\frac{32-11}{7.3 \times 0.58} = 4.9 \text{ micromoles } CO_2 \text{ per gram per hour}$$

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[1]Chester A. Lawson, op. cit., p. 147.

This method is useful for study of respiration rates of different organisms, and of rates of the same organism under different conditions (temperature, O_2 supply, etc.)

Students work individually during this activity but use the same source of water and the same temperature. Put your results on the blackboard and calculate the average for the class. Include the class results with your report and save a copy of this information for a future activity.

Information for the Teacher

This activity can lead to extensive class projects, or to projects by individual students who like quantitative studies.

The method given above is approximate but the author indicates that the error is probably less than ten per cent.

RESPIRATION RATES OF AQUATIC ORGANISMS[1]

ORGANISM	RESPIRATION RATE AT 23° C
Aquatic insects	20 micromoles CO_2/gram/hr
Goldfish	7
Frog tadpole	8
Aquatic newt	8
Aquatic snail	3
Mussel (minus shell)	1 (weight of shell subtracted
Mussel (with shell)	0.5 in computation)

[1] Ibid., p. 148.

EXERCISE VIII. BEHAVIOR OF PLANARIA--ORIENTATION[1]

Directions for the Student

The study of how and why animals behave as they do encompasses a wide field. Areas of behavior investigated in this unit will be limited to the orientation of planaria, the directions in which they move in response to various stimuli.

Orientation is used to include reactions which guide an animal to its normal stance called primary orientation and other reactions which guide the animal into situations that are important to it, such as the reactions which guide the animal into its normal habitat.

An example of primary orientation is the normal position maintained by most fish--dorsal side up and the longitudinal axis horizontal. That their primary or normal position is a reaction should be evident when one considers that dead fish usually float with the ventral surface or the side uppermost.

Taxis is a term indicating "directed orientation reactions."[2] A positive or negative taxic response is used

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[1]Gottfried S. Fraenkel and Donald L. Gunn, The Orientation of Animals, (New York: Dover Publications, Inc., 1961), p. 1-4.

[2]Ibid., p. 10.

for movement directly to or directly away from the stimulus. Transverse orientation is used to describe directed reactions in which the movement is oblique or at an angle to the line joining the organism and the source of stimulation.

Kinesis is orientation through undirected locomotion such as where the speed of movement or frequency of turning is dependent on the intensity of stimulation.

Activity 1. Observe the response of planaria to light.

A. Use an overhead light of the type that the intensity can be changed for different trials. Trace the worm's path for 30 seconds on each trial.

B. Using two lights one to two inches above the bottom of a shallow tray, place planaria equidistant between them. Repeat using lights of unequal brightness. Trace or sketch the paths of five worms as they respond to both situations.

C. Arrange lights in a manner so that planaria react by moving in a square or other preconceived pattern. In these activities is the stimulus light or is it heat? How can you prove your ideas?

Activity 2. Observe the behavior of planaria in response to currents in water.

A. Subject planaria to currents from the side--check the effect on different parts of the body.

B. Subject planaria to currents from in front and currents from the rear.

C. Repeat A and B using water for the currents which has food flavors.

Information for the Teacher

Activity 1. Planaria respond negatively to light. The overhead light will cause an increase in the rate of change in direction. The bright light will cause the most rapid change in direction.

The rate of change of direction can be checked if the path has been traced for thirty seconds. A basal r. c. d. can be checked if a planaria's path is traced for thirty seconds before it has been stimulated by added light.

When placed between two low level lights, the planaria will move away from the lights along a path approximating the perpendicular bisector of an imaginary line joining the two lights. The worm follows a path away from light so that neither eye is receiving more light than the other. Therefore, the planaria's path away from lights of unequal brightness will be closer to the dimmer of the two lights.

Planaria will move in a path that traces a square if

lights can be turned on in sequence. As it moves away from the first light and establishes a route, turn on another light that shines from the side and turn off the first light.

Activity 2. Planaria will turn toward the currents in water. The anterior end of the worm is much more sensitive than the posterior end. Planaria seem to have chemoreceptors in or near the auricles and will turn toward water that has had food soaked in it.

CHAPTER VI

SUMMARY

The purpose of this report was to prepare a guide for the laboratory study of live planaria to implement the course theme, inquiry. This was planned as a six-week laboratory unit for advanced biology in the Marshalltown Community High School.

The research was a document analysis of textbooks, workbooks and laboratory manuals, reference books, and current literature.

General information, research information, and activities were adapted to fit the needs of the course and other activities were synthesized by the investigator from the various sources of information. The material was then organized into a guide to laboratory activities using live planaria for secondary school advanced biology.

The remainder of this field report was organized as follows:

Chapter II describes the advanced biology course in the Marshalltown Community High School and coordinates the laboratory guide, using planaria as the subject for a six-week experimentation unit.

Chapter III is a review of literature presented to establish a background for using planaria as a laboratory animal for secondary school advanced biology.

Chapter IV is a discussion of the work to be done in the laboratory and of the scientific method, followed by general laboratory instructions for the student.

Chapter V is a "Guide to Laboratory Activities Using Live Planaria for Secondary School Advanced Biology" which is the topic of this field report.

The guide has the following exercises: field collecting, care of planaria and observation, ecology, use of a key, morphology, regeneration, respiration, and taxic responses to stimuli in the planaria's environment. Each student activity is followed by pertinent information for the teacher.

The laboratory unit using live planaria will be introduced near the end of the second course division "Resources for Living." It may be advantageous to collect the planaria sooner to insure an adequate supply because the unit will not begin until mid or late November.

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